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Purification of APP C100-FLAG substrate

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An abbreviated version of this protocol was published in eLIFE in Aug 2016

The amyloid-beta forming tripeptide cleavage mechanism of γ-secretase

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Detailed protocol

Purification of APP C100-FLAG substrate

1. Incubate 50 µl BL21(DE3)-competent bacteria with 1 µl plasmid (C100-FLAG in pET22b, 10 ng - 100 ng) for 30 minutes on ice. Heat shock using the hot plate for 45 seconds at 42°C. Incubate on ice for 2 mins. Add 400 µl LB medium warmed to 37°C, mix, and incubate at 37°C for 60 min.

2. Spread the transformation reaction on LB agar plates supplemented with 100 µg/ml ampicillin. Incubate at 37°C overnight.

3. Inoculate 100 ml of LB medium supplemented with 100 µg/ml ampicillin in a 500 ml flask, with a single colony from the plate. Grow overnight at 37°C with agitation at 200 rpm in an incubated shaker.

4. Add 5 ml of the 100 ml overnight culture (pre-culture) to 500 ml (in a 2 liter flask) of Terrific broth supplemented with 100 µg/ml ampicillin (use as many flasks as desired depending on the amount of protein needed). Grow at 37°C with agitation at 250 rpm until the OD600 reaches 0.8. Keep 1 ml of culture as control expression before induction.

5. Add IPTG to a final concentration 1 mM and incubate for 3 h at 37°C with agitation at 250 rpm.

6. Cells are then pelleted at 8,000 g for 20 min.

7. Re-suspend cell pellets in lysis buffer containing 50 mM HEPES, pH 7.0, 1% Triton X-100 detergent supplemented with protease inhibitor cocktail. Cells are lysed by French press.

8. Centrifuge the solution at 100,000 g to obtain clear lysate.

9. Isolate FLAG-tagged APP-C99 immunoprecipitation for 3 h at 4°C with anti-FLAG M2 beads from Sigma.

10. Elute the FLAG-tagged APP-C99 with 100 mM glycine, pH 2.5, 0.25% NP40 prior to being neutralized with Tris buffer and stored at -80。C.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Bolduc, D. , Wolfe, M. and Selkoe, D. (2020). Purification of APP C100-FLAG substrate. Bio-protocol Preprint. bio-protocol.org/prep276.
2. Bolduc, D. M., Montagna, D. R., Seghers, M. C., Wolfe, M. S. and Selkoe, D. J.(2016). The amyloid-beta forming tripeptide cleavage mechanism of γ-secretase. eLIFE. DOI: 10.7554/eLife.17578

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